PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
A2469PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI2003/000544	04.07.2003	05.07.2002
International Patent Classification (IPC) or national classification and IPC		
C12Q 1/68		
Applicant		
Valtion Teknillinen Tutkimuskeskus et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☒ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.01.2004	11.10.2004
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/[redacted]003/000544

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-46 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 48, 49, 51-52 received by this Authority on 13.09.2004

pages* 47, 50 received by this Authority on 05.10.2004

☒ the drawings:

pages 1-18 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000544

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority is considered valid. Document WO02055734 is therefore not considered herein.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/[redacted]003/000544

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-35	YES
	Claims		NO
Inventive step (IS)	Claims	1-35	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:

D1) US5807682
D2) WO9937663 A1
D3) US6268144 B1
D4) Nucleic Acids Research, Volume 27, no. 23, 1999, Olejnik J. et al: "Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS", pp. 4626-4631
D5) Anal. Chem., Volume 73, 1 May 2001, Isola N. R. et al: "MALDI-TOF Mass Spectrometric Method for Detection of Hybridized DNA Oligomers", pp. 2126-2131
D6) US6136531
D7) US5633134
D8) US6395486 B1

The present application relates to methods and kits for quantitative determination of ribopolynucleotides in mixtures of analyte polynucleotide sequences. According to the applicant, the problem to be solved is how to get polynucleotide sequences having approximately the same number of nucleotides distinguishable. The problem is to provide a method for a quantitative determination of several different polynucleotides simultaneously.

D1 shows a method for detecting multiple selected sequences. A plurality of probes is reacted with the target polynucleotides. The target polynucleotide may include several nucleic acid molecules such as RNA or DNA. The probes all have about the same length or are identical in length and thus can be hybridized with target sequences with about the

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

same hybridization kinetics and thermodynamics. Each probe includes a binding polymer having a probe-specific sequence, a polymer chain which imparts to the probe a distinctive ratio of charge/translational frictional drag, and a reporter. The target and hybridized probes are then captured (immobilized) by adding a solid support which is complementary to a region of the target polynucleotide. Bound probes are released and the probes are fractionated by e.g. capillary electrophoresis in a non-sieving matrix to identify target sequences. The probes can further include a second binding polymers allowing primer initiated polymerisation. See fig. 20A-20C, col. 3 lines 34-43, col. 3 line 66-col. 4 line 3, col. 4 lines 33-42, col. 5 line 1-col. 6 line 5, col. 7 lines 1-5, col. 12 lines 28-39, col. 15 lines 36-42, col. 20 line 22-col. 21 line 8 and claims 1-4.

D2 shows a quantitative method for detecting a specific nucleic acid in a population, collection, of nucleic acids. The method is based on the use of a tagged oligonucleotide (a detector primer) that is complementary to at least a portion of a specific nucleic acid in the population. Detector primers are annealed to the target nucleic acids to form a mixture of hybridized and non-hybridized oligonucleotides. Each detector primer includes a specified oligonucleotide sequence attached to a tag via a cleavable linker. A polyA-tract on target RNA is used to separate polyA-containing RNA molecules bound to the detection primers from non-hybridized detection primers and nucleic acids lacking a polyA region. The linker is cleaved to release the tags and the identity of the tag is determined. The presence and the absolute amount, and/or the relative amount of a given tag, reflects the amount of the target nucleic acid present in the sample. The tag can be detected using mass spectrometry and the tags can be amino acids or polymers. The hybridization can be done in solid phase or solution and is typically conducted with molar excess of detector oligonucleotide to target. The detector oligonucleotide can be provided with a second tagged oligonucleotide (selector oligonucleotide) acting to increase the specificity of detection. The selector and detector oligonucleotides can be used as primers. The tag on the

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Continuation of: Box V

selector oligonucleotide can be a fluorescent dye that would allow the use of optical sorting to identify tagged oligonucleotides. Each oligonucleotide linked to a tag can be distinguished by the way in which the tag is attached to the oligonucleotide. Distinguishable tagged oligonucleotides and kits thereof are included. See p. 3 lines 11-p. 4 line 25, p. 5 lines 7-p. 6 line 19, p. 7 lines 13-21, p. 11 line 19-p. 12 line 6, p. 12 line 12-p. 13 line 5, and claims 6-7 and 12.

D3 shows a mass-spectrometry-based process for detecting nucleic acid sequences in a biological sample. A number of target sequences are immobilized by using capture sites interacting with complementary immobilized capture sequences. The use of biotin and streptavidin to immobilize and separate hybridization sequences is discussed. Detection is achieved by employing mass differentiated detector oligonucleotides, which can be mass differentiated by their sequences or by mass modifying functionalities. The method can be used to detect or quantify nucleic acid sequences that are specific to infectious organisms. A number of mass-modifying functionalities such as oligonucleotides and peptides are mentioned. The use of probes acting as primers is mentioned. See col. 4 lines 44-53, col. 6 lines 42-63, col. 12 line 41-col. 13 line 64, col. 14 line 21-col. 15 line 52, col. 17 lines 4-12 and claims 3, 5 and 15-16.

D4 shows a method for detecting DNA wherein photocleavable peptide-DNA conjugates are used and detected using MALDI-MS. The DNA portion of the conjugate acts as a hybridization probe, whereas the peptide is photoreleased and can serve as a mass tag to identify a unique target DNA sequence. See abstract, p. 4627 Figure 1 and p. 4630-4631 "Discussion".

D5 shows a method wherein total genomic DNA of bacteriophages is identified by hybridization of species-specific oligonucleotide probes. Multiple-probe hybridization is resolved by mass spectrometry. Solution hybridizations are made and mass tags are used for detection of multiple probes simultaneously. See abstract, p. 2127 left col. paragraph 1 and p. 2130 left col. paragraph 2-p. 2131.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D6 shows a method of quantitatively detecting specific nucleotide sequences characterized in that nucleic acid id hybridized in solution with a probe. The nucleic acid is then immobilized on solid phase and the amount of bound hybrid is detected. See abstract, Figure 7 and col. 2 lines 27-52.

D7 shows a method for testing large number of individuals for the presence or absence of mutations in one or multiple genes using allele specific probes. The probes used in a pool to detect multiple mutations in the same hybridization reaction are all of approximately the same length. The probes are labelled with phosphor. See col. 2 lines 27-37, col. 3 lines 13-17, col. 4 lines 8-11 and col. 5 lines 20-38.

D8 shows a method for detecting one or more target nucleic acid sequences present in a sample. A sample is contacted with probes, where each probe includes a target-specific portion and a tag. Exemplary tags and/or tag complements include antibodies and associated antigen or hapten, receptors and associated ligands, avidin (or streptavidin) and biotin, and polynucleotide sequences and their complementary sequences. The hybridized probe is treated to form a modified probe, where the treatment is effective to distinguish probes that have bound to the target nucleic acid. The probes are contacted with mobility-modifiers to form a probe/mobility modifier complex, each mobility modifier having a tag complement for binding selectively to the tag of an associated probe. Finally, the probe/mobility modifier complex is analyzed using a mobility-dependent technique. See abstract, col. 2 lines 8-25, col. 4 lines 55-61 and Fig. 4A-D.

D1 is considered to represent the closest prior art. In present claim 1 it is stated that the analyte nucleotide has at least one affinity tag. This "affinity tag" corresponds to the sequence in D1 used to immobilize the target and hybridized probe.
Consequently, the method according to claim 1 differs from D1 in that the invention according to present claim 1 relates to the analyte being ribopolynucleotides. D1 suggests that the method is applicable to both DNA and RNA but it does not describe specifically the quantification of ribopolynucleotide sequences. The instability of RNA and problems related to quantification of RNA is well known to the skilled person, but is not mentioned in D1. The examples in D1 do not show quantification of RNA.

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Supplemental Box

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Continuation of: Box V

Furthermore, the method according to present claim 1 is quantitative, whereas the method in D1 is qualitative. D1 relates to detecting the presence and absence of target polynucleotides using probe compositions for detecting selected sequences in a target polynucleotide. In the present application, probes are analyzed and quantified after separation from the sample sequences whereas D1 only discloses that the sample nucleotide sequence fragments are separated according to their size.
In the invention according to claim 1, resolution enabling tags change the mass charge of the probes which therefore can be separated in a sieving medium. In contrast, the probes of in D1 are provided with non-charged polymer chains which enable them to move with different rates in a non-sieving medium. In D1, the fact that the charge/translation frictional drag providing polymer chain may be attached to the probes after hybridization in order to make them distinguishable by fractionation by electrophoresis in a non-sieving medium seems to demonstrate that the detection is not intended to be quantitative. Thereto, the method according to claim 1 of the present application differs from D1 in that in D1 it is not explicitly stated that the probes are present in a surplus in relation to analyte polynucleotide.

The effect of the differences mentioned above seems to be that RNA can be accurately quantified.

The problem to be solved by the present invention may therefore be regarded as to provide a method for accurate quantitative determination of target ribopolynucleotides.

There are no indications in the prior art that would lead a person skilled in the art to apply the features from the cited documents and thus arrive at the invention as defined in claim 1. Therefore, the invention according to claims 1-35 fulfils the requirements of novelty and inventive step.

In D2 it is suggested on pages 4 and 5 that both RNA and DNA can be quantitatively measured as the cleavable tag, which is

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

said to reflect the amount of target nucleic acid present in the sample. No experimental evidence is shown. In an alternative method which addresses the problem of instability of RNA, the mRNA is not measured directly, but as cDNA which has been reversely transcribed.
In D2, the oligonucleotide probes used seem to have the same length or approximately the same length (see figure 1) and they are present in a surplus.
However, in the method of present claim 1, the oligonucleotide probe with tag is measured and quantified, whereas in D2, a cleavable tag which can be released from the probe is measured and quantified.
The method in present claim 1 relates to the target being ribopolynucleotide sequences. It is suggested in D2 that both RNA and DNA can be quantitatively measured as the cleavable tag which is said to reflect the amount of the target nucleic acid. However, this does not seem highly credible in view of the lack of experimental evidence that a method requiring additional separation steps and chemical release would be as quantitative as the method of the present application. This is especially true in view of the instability of RNA. An alternative method shown in D1 addresses the instability problem. In this method the mRNA is not measured directly, but as cDNA, leading to losses of RNA and that the RNA cannot be said to be accurately quantified.

The detector oligonucleotides of D3 have approximately the same number of hybridizing nucleotides (see Figure 3) and are made distinguishable by providing mass modifying tags. The capture sites used in D3 (the use of biotin is mentioned) correspond to the affinity tag of present claim 1.
However, in the present application solution hybridization is preformed. In D3, target polynucleotides are immobilized to a solid support. The present application relates to quantification of RNA, whereas D3 relates to determination of genomic DNA (if RNA is detected, it is measured indirectly as cDNA).

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/[redacted]003/000544

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO02055734	18/07/2002	10/01/2002	10/01/2001

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

The wording of present claims 17 and 18 is not entirely clear. Claim 17 seems to relate to a method according to claim 1 characterized in that an assessment of variations in the amounts of polynucleotides is made by providing a set of multiple kits. However, with the present wording, claim 17 seems to lack a verb.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000544

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Present claim 1 is not clear and concise (see PCT Art. 6) since the expression "approximately", used when describing the number of nucleotides in the probe, is not clearly defined.

2. The method according to claim 1, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide transcripts or their subgroups in an individual organism, the soluble polynucleotide probes are designed from species or group-specific ribopolynucleotide sequences hybridizing with selected more or less conserved or hypervariable regions from intragenomic sequences specific for subgroups, species, subspecies of transcripts expressed in the organism.

3. The method according to claim 2, **characterized** in that the analyte polynucleotide sequences isolated from the sample comprising the mixed target population is messenger RNA (mRNA).

4. The method according to claim 1, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide sequences representing individual organisms or subpopulations thereof in a target population, the soluble deoxyribopolynucleotide probes are designed from species or group-specific ribopolynucleotide sequences hybridizing with a selected more or less conserved or hypervariable region from intragenomic sequences specific for and/or representing different phylogenetic levels allowing the identification of subgroups, species, subspecies within the mixed target population.

5. The method according to claim 4, **characterized** in that the ribopolynucleotide analytes isolated from the sample comprising the mixed target population comprise ribosomal RNA.

6. The method according to claim 1, **characterized** in that the resolution enabling tag may simultaneously act as a tracer, affinity and/or primer tag.

7. The method according to claim 1, **characterized** in that the resolution enabling tag is separatable in a sieving medium.

8. The method according to claim 7, **characterized** in that the resolution enabling tag which additionally may act as an affinity and/or primer tag is an oligonucleotide residue.

9. The method according to claim 7, **characterized** in that the resolution enabling tag which additionally may act as an affinity tag and/or a tracer tag is an amino acid or a peptide.

10. The method according to claim 7, **characterized** in that the resolution enabling tag which additionally may act as a tracer tag is selected from a group consisting of labels recordable by fluorescence, luminescence, infrared absorption, electromagnetic properties, radioactivity and enzymatic activity.

11. The method according to claim 1, **characterized** in that the preset optional number of soluble polynucleotide probes in the pool is more than one preferably more than five, most preferably more than ten.

12. The method according to claim 1, **characterized** in that the amount of the individual, quantitatively captured and released polynucleotide probes is recorded with a fully or partly automized recording system, which is selected based on the applied resolution enabling tags.

13. The method according to claim 12, **characterized** in that the recording system is selected based on resolution enabling tags and comprises mass spectrometry, electrophoretic or chromatographic techniques.

14. The method according to any of claims 1-13, **characterized** in that the amount of the quantitatively recovered primer tagged probes are released and subsequently amplified and optionally tracer tagged before, during or after the PCR-reaction and thereafter recorded with a recording system selected based on the resolution enabling tags.

15. The method according to claim 14, **characterized** in that the primer is a universal primer.

16. The method according to claim 1, **characterized** in that the polynucleotide probes are stable DNA fragments, synthetic or recombinant polynucleotide sequences or modified polynucleotide sequences.

17. The method according to claim 1, **characterized** in that a comparative, quantitative assessment of variations in the amounts of individual polynucleotide sequences or organisms and subgroups thereof in a population or mixture of polynucleotide sequences by providing a set of multiple test kits, at least one test kit for each sample to be compared,

21. The use according to claim 19, **characterized** in that the polynucleotide analytes isolated from the sample comprising the mixed target population comprise messenger RNA (mRNA).

22. The use according to claim 19, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide sequences representing individual organisms or subpopulations thereof in a target population, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable region from intragenomic sequences specific for and/or representing different phylogenetic levels allowing the identification of subgroups, species, subspecies within the mixed target population.

23. The use according to claim 22, **characterized** in that the polynucleotide analytes isolated from the sample comprising the mixed target population comprise ribosomal RNA.

24. The use according to claim 19, **characterized** in that the resolution enabling tag may simultaneously act as a tracer, affinity or primer tag.

25. The use according to claim 24, **characterized** in that the resolution enabling tag is separable in a sieving medium.

26. The use according to claim 25, **characterized** in that the resolution enabling tag which additionally may act as an affinity tag and/or primer tag is an oligonucleotide residue.

27. The use according to claim 25, **characterized** in that the resolution enabling tag which additionally may act as an affinity tag and/or a tracer tag is an amino acid or a peptide.

28. The use according to claim 25, **characterized** in that the resolution enabling tag which additionally may act as a tracer tag is selected from a group consisting of labels recordable by fluorescence, luminescence, infrared absorption, electromagnetic properties, radioactivity and enzymatic activity.

29. The use according to claim 19, **characterized** in that the preset optional number of soluble polynucleotide probes in the pool is more than one preferably more than five, most preferably more than ten.

30. The use according to claim 19, **characterized** in that the soluble pools of polynucleotide probes are placed in wells on a microtiter plate.

31. The use according to claim 19, **characterized** in that the polynucleotide probes are stable DNA fragments, synthetic, recombinant or modified polynucleotide sequences.

32. The use according to claim 19, **characterized** in that for a comparative, quantitative assessment of variations in the amounts of individual polynucleotide sequences or organisms and subgroups thereof in a population or mixture of polynucleotide sequences comprises a set of test kits, wherein at least one identical test kit having identical pools of polynucleotide probes for each sample to be compared.

33. The use according to claim 32, **characterized** in that each individual test kits in the set of multiple test kits is provided with tracer tags, which are distinguishable from each other by the emitted signal.

34. Use according to claim 32 for assessing hygienic conditions and epidemiologic situations, effects of external stimuli or treatment modalities on a microbial population.

35. The use according to claim 34, wherein the external stimulus or treatment is selected from a group consisting of treatment with antibiotics or hygienic measures.

Claims

1. A method for determination of amounts or relative proportions of more than one individual polynucleotide sequence or subgroups thereof in a polynucleotide mixture using a quantitative affinity aided solution hybridization in combination with size- or mass-based fractionation for obtaining resolution, **characterized** in that the method comprises the consecutive steps of:

(a) providing, one or more organized pools with a preset optional number of soluble polynucleotide probes, each probe being complementary to an individual target ribopolynucleotides in the sample, being present in a molar excess as compared to the analyte polynucleotide sequences, and having approximately the same number of hybridizing nucleotides, which probes are made distinguishable by providing said polynucleotide probes with one or more resolution enabling tags, which change the size or mass and thereby provide the polynucleotide probes with different mobilities in fractionation, separation or recording systems without disturbing the hybridization or capturing reaction;

(b) providing the analyte polynucleotide sequences isolated from a sample comprising a mixture of target ribopolynucleotide sequences with at least one affinity tag; and thereafter

(c) performing steps (i) and (ii) simultaneously, or sequentially; in the order (i) and (ii), wherein the steps (i) and (ii) comprises

(i) allowing a hybridization reaction to take place between the molar excess of soluble polynucleotide probes from step (a) and the analyte ribopolynucleotide sequences from step (b) leading to a quantitative formation of soluble hybrids;

(ii) recovering the hybrids, which have been quantitatively formed in step (i) by capturing said hybrids quantitatively on a separation aiding tool provided with the affinity pair of the affinity tag of the analyte polynuclotides;

(d) quantitatively releasing the polynucleotide probes in an unmodified form from the hybrids captured to separation aiding tool;

(e) separating and recording the amount or relative proportions of distinguishable probes, the amount of which corresponds to the amount of complementary target ribopolynucleotide sequences in the mixture of analyte ribopolynucleotides in the sample.

wherein each of said test kits are provided with one or more identical organized pools with a preset optional number of soluble polynucleotide probes, each probe being DNA complementary to an individual target ribopolynucleotide sequence in the sample, being present in a molar excess as compared to the target polynucleotides in the samples, and having an indistinguishable number of hybridizing nucleotides, which probes are made distinguishable by providing each polynucleotide probe with one or more resolution enabling tags, which change the size or mass and thereby provide the polynucleotide probes with different mobilities in the fractionation, separation or recording systems without disturbing the hybridization or capturing reaction, each pool of polynucleotides probes being placed in an organized manner in their own vessels, which are separate or joined together.

18. The method according to claim 17, **characterized** in that the individual test kits, wherein the resolution enabling tag is not a tracer tag, a set of multiple test kits is provided with tracer tags each being distinguishable from the other by the emitted signal.

19. The use in any method according to claims 1-18 of a test kit, **characterized** in that the test kit comprises one or more organized pools, with a preset optional number of soluble polynucleotide probes, each probe being complementary to an individual target ribopolynucleotide sequence in the sample, being present in a molar excess as compared to the analyte ribopolynucleotides in the samples, and having approximately the same number of hybridizing nucleotides, which probes are made distinguishable by providing each polynucleotide probes with one or more resolution enabling tags, which change the size or mass and thereby provide the polynucleotide probes with different mobilities in the fractionation, separation or recording systems without disturbing the hybridization or capturing reaction, each pool of polynucleotides probes being placed in an organized manner in their own vessels, which are separate or joined together.

20. The use according to claim 19, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide transcripts or their subgroups in an individual organism, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable region from intragenomic sequences specific for subgroups, species, subspecies of transcripts expressed in the organism.